Physiological functions of biliary lipid secretion

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CHAPTER 9

DOWN-REGULATION OF INTESTINAL SCAVENGER RECEPTOR CLASS B, TYPE I (SR-BI) EXPRESSION IN RODENTS UNDER CONDITIONS OF DEFICIENT BILE DELIVERY TO THE INTESTINAL LUMEN

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Submitted

ABSTRACT

Background and Aims: Bile components play a crucial role in intestinal cholesterol absorption. Scavenger receptor class B , type 1 (SR-BI) is expressed in the intestine of rabbits and has been suggested to be involved in the absorption of dietary cholesterol. The aim of this study was to determine whether intestinal SR-BI expression is affected in animal models with altered bile delivery to the intestine and impaired cholesterol absorption. *Methods*: SR-BI protein and mRNA levels were determined in proximal and distal intestine from control, bile duct-ligated and bile-diverted rats and from control, bile duct-ligated mice and mdr2 P-glycoprotein-deficient mice that produce phospholipid/cholesterol free bile. Cholesterol absorption efficiency was quantified by a dual isotope approach. *Results*: SR-BI was present at the apical membrane of enterocytes in control rats and mice and more abundant in proximal than in distal segments of the intestine. In bile duct-ligated animals, protein levels of SR-BI were virtually absent and mRNA levels are decreased by ~ 50%. Bile-diverted rats and *Mdr2*$^{(-/-)}$ mice showed decreased levels of intestinal SR-BI while mRNA levels remain unaffected. Cholesterol absorption was reduced by > 90% in bile duct-ligated and bile-diverted animals whereas *Mdr2*$^{(-/-)}$ mice show a ~50% reduction in absorption efficiency. *Conclusions*: This study shows that SR-BI is expressed at the apical membrane of enterocytes mainly in the upper intestine of rat and mouse and that bile components (cholesterol, phospholipids) play a role in post-transcriptional regulation of its expression. Factors associated with cholestasis, e.g., elevated plasma bile salts and cholesterol, appear to be involved in transcriptional regulation of intestinal SR-BI expression. The role of SR-BI in the cholesterol absorption process remains to be defined.

INTRODUCTION

Cholesterol absorption from the intestine is a key process in the regulation of whole body cholesterol homeostasis [1,2]. Intestinal cholesterol can be of endogenous (bile, intestinal cells) or of exogenous (diet) origin. The sterol is taken up by enterocytes in its free form, esterified, packaged into chylomicrons by epithelial cells and secreted in to the lymph [3,4]. After (partial) hydrolysis of their triglyceride content, the resulting cholesterol-rich chylomicron remnants are rapidly cleared by the liver [5]. Bile plays a crucial role in the cholesterol absorption process [6-9]. Bile components provide the solubilizing vehicles for cholesterol by formation of mixed micelles, consisting of bile salts, phospholipids and cholesterol [10,11]. Several studies [3,7,12] have shown that bile salts are essential herein but available data indicate that the individual micellar components are taken up independently [13-15]. Recently, Sehayek *et al.* [16] proposed that biliary cholesterol secretion (co-) regulates dietary cholesterol absorption in mice, but the underlying mechanisms have remained unclear. It is of importance to note that, in humans on a western-type diet, bile delivers two to three times more cholesterol to the intestinal lumen on a daily basis than does the diet [1,4]. In rodents fed standard lab chow, cholesterol entering the intestine is almost exclusively (> 80%) of biliary origin. The sequential steps of intestinal cholesterol absorption have been studied extensively [4] but the actual mechanism(s) responsible for uptake by the intestinal cells from the lumen is not known. It has been suggested that cholesterol uptake is energy-independent and reflects passive diffusion down a concentration gradient [17]. Other studies indicate that cholesterol uptake is a protein-mediated process. Turnhofer *et al.* [18] provided evidence to suggest that uptake of cholesterol may be catalyzed by an intrinsic membrane protein. Recently, Landschulz *et al*. [19] have reported that a member of the scavenger receptor family, scavenger receptor, class B, type I (SR-BI) is expressed in the intestine of rodents. Furthermore, Hauser *et al.* [20] have recently shown SR-BI is present in the small intestinal brush border of rabbits and, in *in vitro* experiments, it appears to facilitates uptake of free cholesterol from bile salt micelles and phospholipid vesicles into brush border membrane vesicles. SR-BI has recently been identified as a High Density Lipoprotein (HDL) receptor [21,22] involved in selective uptake of cholesterylesters from HDL by various organs, including liver and steriodogenic tissues [19,23,24]. In addition, SR-BI facilitates both non-lipoprotein cholesterol uptake [25] as well as free cholesterol efflux from cultured cells [26]. So far, however, nothing is known about regulation of intestinal SR-BI expression and whether alterations in its expression are actually associated with changes in cholesterol absorption efficiency

In view of the role of bile constituents in cholesterol absorption and the proposed role of SR-BI in this process, we have investigated whether bile components influence intestinal SR-BI expression in rats and mice. Bile delivery into the intestine is completely blocked in bile duct-ligated rats and mice leading to accumulation of bile components in liver and plasma and to elevated plasma cholesterol levels [27-29]. Long-term bile diverted rats also experience a complete absence of bile in the intestinal lumen but without the potentially interfering consequences of cholestasis [30]. To differentiate between actions of biliary bile salts and lipids, we used mdr2 P-Glycoprotein deficient ($Mdr2^{(-/-)}$) mice [31], in

which biliary phospholipids and cholesterol secretion is strongly impaired whereas bile salt secretion is unaffected [32].

MATERIALS AND METHODS

Animals: Male Wistar rats (~300g) from the breeding colony of the University of Groningen were used. Bile-diverted rats were prepared as described previously [30] by providing the animals with a permanent silastic bile duct catheter. Bile was diverted for 7 days prior to the actual experiments. Bile duct ligation was performed in rats and FVB mice under halothane anesthesia. These animals were used 5 days after surgery. Mice homozygous for disruption of the multidrug resistance gene-2 (*Mdr2*$^{(-/-)}$) and control (*Mdr2*$^{(+/+)}$) mice of the same FVB-background were obtained from the breeding colony at the Animal Facility of the Academic Medical Center, Amsterdam. All mice were ~3 months of age. Animals were housed in a temperature- and light- (12 hours light cycle) controlled environment and were fed a standard lab-chow *ad libitum*. The experimental protocols were approved by the ethical committee on animal testing, University of Groningen, The Netherlands.

Intestinal SR-BI protein levels: Animals were anesthesized with Halothane and the small intestines were rapidly removed and divided in two equal parts and flushed with phosphate-buffered saline containing protease inhibitors (Complete®, Boehringer Mannheim, Mannheim, Germany). Intestinal mucosa was scraped for homogenization in buffer containing 250 mM sucrose, 10 mM Tris-base (pH 7.4) and protease inhibitors (Complete®). From these homogenates, brush border membrane (BBM) fractions were isolated by calcium precipitation as described by Schmitz *et al*. [33]. In short, homogenates were mixed with buffer (50 mM sucrose, 2 mM Tris, pH 7.4) containing $CaCl_2$ (final concentration 10 mM). The mixture was incubated for 15 min on ice and centrifuged 2000 x g (Optima ^{TLX} Tabletop Ultracentrifuge, Beckman, Fullerton, CA, USA) for 15 min at 4^{o}C. The supernatant was centrifuged at 20,000 x g for another 15 min at 4^{o}C. The remaining pellet (P2 or BBM) was resuspended in homogenization buffer. Alkaline Phosphatase activity, used as a marker for enrichment of the BBM [33], was determined using the method described by Keeffe *et al*. [34].

After determination of total protein concentrations, equal amounts of protein, i.e., 5 mg for BBM and 30 mg for total membrane fractions, were loaded on a 4-15% gradient SDS-Page gel (BioRad, Hercules, USA) and electophoresed at 100 V. Proteins were blotted onto nitrocellulose membranes (BioRad, Hercules, USA) by tankblotting (300 mA, 2h). Nitrocellulose membranes were blocked overnight in 5% skim milk powder solution in Tris buffered saline (TBS) containing 0.1 % Tween and subsequently incubated with the primary antibody (rabbit polyclonal anti-murine SR-BI, 495 [21]) diluted 1:10.000 in 5% skim milk powder in TBS-Tween for 2h at room temperature. After washing a secondary antibody, anti-rabbit Ig linked to Horse Radish Peroxidase (Amersham, Little Chalfort, UK), diluted 1:1000 in 5% skim milk powder in TBS-Tween, was added for another hour. Detection was performed using ECL (Amersham) according to the manufacturers instructions. Liver total membrane fractions used for comparison were prepared as described [35]. For Western blot analysis of liver homogenates, ~ 1 mg of total membrane proteins were separated by SDS-Page. The ß-subunit of Na+/K+-

ATPase constitutive expression levels were used as a reference signal detected with, antibodies kindly provided by Dr. Wilbert Peters (Nijmegen, The Netherlands).

Deglycosylation of BBM and liver homogenate proteins was performed by incubation with N-deglycosylase (PNGase kit, New England Biolabs, Beverly, MA) following the manufacturer's instructions.

Imunohistochemistry and confocal laser analysis: Liver and small intestinal sections were collected, immediately frozen in liquid isopentane and 4 mm slices were cut of these tissues and fixed with acetone. The first antibody, anti-SR-BI (rabbit anti-murine SR-BI in 1% BSA/PBS) [21] was incubated followed by washing with PBS. Endogenous peroxidase was inhibited using 30% methanol, 0.3% H2O2 and detection was done with peroxidase-linked rabbit anti-guinea pig-Ig (Dako A/S, Glostrup, Denmark) with an amplification step using goat anti-rabbit-Ig (Dako A/S). 3-Amino-9-ethylcarbozole (Sigma, St. Louis, MO) was used as a substrate and tissue was counterstained with haematoxylin. For confocal scan microscopy Detection was performed using FITC-linked anti-rabbit Ig.

Intestinal SR-BI mRNA levels: Total RNA was isolated from intestinal tissue a combination of the TRIzol Reagent (GIBCO BRL, Grand Island, NY) and the SV Total RNA isolation system (Promega, Madison WI, USA) according to the manufacturer's instructions. Single stranded cDNA was synthesized from 4.5 mg RNA and subsequently subjected to polymerase chain reactions (PCR) using specific primers sets for rat and mouse HMG-CoA reductase (HMGR) (sense primer: 5'-GACACTTACAATCTGTATGATG-3'; antisense primer: 5'-CTTGGAGAGGTAAAACTGCCA-3'), SR-BI (sense primer: 5'-CTCATCAAGCAGCAGGTGCTCA-3'; antisense primer: 5'-GAGGATTCGGGTGTCATGAA-3') and b-actin (sense primer: 5'-AACACCCCAGCCATGTACG-3'; antisense primer: 5'-ATGTCACGCACGATTTCCC-3'). The PCR products were ran on 2.5% agarose gels and stained with ethidium bromide. Images were taken using a CCD video camera of the ImageMaster VDS system (Pharmacia, Upsulla, Sweden).

Intestinal cholesterol absorption: Intestinal cholesterol absorption was determined using the dual-isotope ratio method of Zilversmit and Hughes [36], as recently modified for use in rodents by Turley *et al*. [37]. In short, animals were given an intravenous dose of ^{3}H-labeled cholesterol (0.54 mg/5.0 mCi for rats and 0.27 mg/2.5 mCi for mice) dissolved in Lipofundin S (Lipofundinâ S 20%, B. Braun Melsungen AG, Melsungen, Germany) and, at the same time, an oral dose of ^{14}C-labeled cholesterol (0.18 mg/2.5 mCi for rats and 0.07 mg/1.0 mCi for mice) dissolved in medium-chain triglyceride oil. After 48 hours, a blood sample was drawn by tail bleeding and the ratio between ^{14}C-labeled and ^{3}H-labeled cholesterol was determined in plasma by scintillation counting. Intestinal cholesterol absorption was calculated as described previously [35].

Biochemical analysis: Protein contents of tissue total membrane fractions and BBM fractions were determined using the Lowry method [38]. Plasma cholesterol levels were determined using a commercially available kit (Boehringer Mannheim, Mannheim, Germany). Plasma amino transaminases (ALT and AST), alkaline phosphatase and total bilirubin were determined by standard clinical chemical

procedures. Contents of cholesterol in intestinal homogenates were determined after lipid extraction [39] as described previously [40].

Statistical analysis: Results are presented as means – standard deviations for the number of animals indicated. Differences between three experimental groups were determined by one-way ANOVA analysis, with posthoc comparison by Newmann Keuls t-test [41]. Differences between two experimental groups were determined using Mann Whitney U test [41]. Level of statistical significance of the difference was set at $p < 0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL, USA)

Results

Plasma biochemical analyses: In order to evaluate the role of bile components on SR-BI expression, we used bile-diverted rats, bile duct-ligated rats and mice and *Mdr2*$^{(-/-)}$ mice. Plasma bilirubin, AST, ALT and cholesterol levels were markedly increased in bile duct-ligated rats when compared to control rats, while no changes were noted in bile-diverted rats (**Table 1**). Body weight and food intake were similar for all three groups (data not shown). Transaminases and bilirubin were clearly also elevated in *Mdr2*$^{(-/-)}$ mice as compared to wildtype mice, as previously described [32,42]. Bile duct ligation in wild type mice provoked a more pronounced increase in these parameters. Plasma cholesterol was reduced in *Mdr2*$^{(-/-)}$ mice [35] and increased in cholestatic mice.

Table 1*: Plasma biochemical analysis of bile-diverted rats duct-ligated rats and mice and Mdr2*$^{(-/-)}$ *mice.*

Species	*Experimental condition*	*AST (U/L)*	*ALT (U/L)*	*Bilirubin (mmol/L)*	*Cholesterol (mmol/L)*	*Cholesterol absorption (%)*
Rat	*control*	*59 – 9*	*34 – 6*	*7.3 – 2.3*	*1.9 – 0.2*	*57 – 10*
	BDL	*475 – 261*[a]	*131 – 80*[a]	*181 – 42*[a]	*6.7 – 4.3*[a]	*2 – 1*[a]
	BD	*66 – 4*	*48 – 7*	*10.0 – 5.3*	*1.6 – 0.1*	*5 – 2*[a]
Mouse	*control*	*78 – 24*	*29 – 6*	*3.7 – 0.5*	*3.5 – 0.8*	*70 – 13**
	Mdr2$^{(-/-)}$	*203 – 64*[b]	*217 – 68*[b]	*5.7 – 0.7*[b]	*1.5 – 0.9*[b]	*42 – 8*[b]*
	BDL	*409 – 32*[b]	*372 – 124*[b]	*138 – 31*[b]	*5.6 – 2.1*[b]	*2 – 1*[b]

All results are given as mean – SD (n = 3-5). Plasma AST, ALT, bilirubin and cholesterol were determined by standard clinical chemical procedures. Cholesterol absorption was determined via the dual isotope method [37]. BDL = bile duct-ligated; BD = bile diversion; Mdr2$^{(-/-)}$ *= mdr2 P-glycoprotein-deficiency.*
[a] *significantly different from control rats, $p < 0.05$.*
[b] *significantly different from control mice, $p < 0.05$*
published previously in reference [35].

Intestinal cholesterol absorption: Cholesterol absorption, as quantified by the the dual isotope method [36,37], was strongly decreased in bile duct-ligated and bile-diverted animals (**Table 1**). As reported previously [35], the absence of biliary phospholipid and cholesterol in *Mdr2*$^{(-/-)}$ mice was associated with a ~40% reduction of cholesterol absorption efficiency. Total cholesterol content of the intestinal mucosa did not differ between control, bile-diverted and bile duct ligated rats, with values ranging from 8.5 - 10.5 nmol/mg protein and 11-12 nmol/mg protein for proximal and distal parts of the intestine, respectively. Similar results

were observed in *Mdr2*$^{(-/-)}$ or BDL mice, ranging 14 - 18 nmol/mg protein and 19 - 23 nmol/mg protein for proximal and distal parts of the intestine, respectively.

Intestinal SR-BI protein expression: Protein levels of SR-BI were assessed by Western analysis in brush border membrane (BBM) fractions of proximal and distal segments of rat intestine. As previously reported [19], the protein band detected in proximal BBM was ~ 78 kDa in size, i.e., ~ 4 kDa less than in hepatic total membrane fractions (~ 82 kDa) (Figure 1). Both hepatic and intestinal membrane fractions showed a SR-BI immunoreactivity at ~ 60 kDa after N-deglycosylase treatment (**Figure 1**), indicating tha presence of a similar intact SR-BI amino acid chain in both tissues. The relative abundance of SR-BI was evidently lower in intestine than in liver of rats. Similar results were obtained for mouse intestine (data not shown).

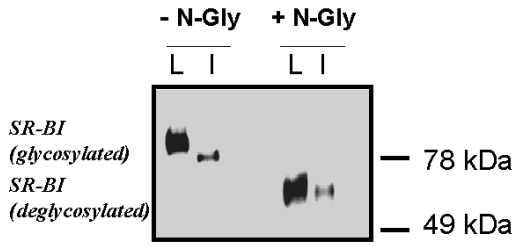

Figure 1*: Western blot of SR-BI in hepatic plasma membranes and intestinal brush border membranes isolated from rats. Deglycosylation was performed as described in the Material and Method section. N-Gly = N-deglycosylase, L = Liver, I = intestine. The amount of protein loaded was 1 mg for hepatic and 5 mg for intestinal preparations. The molecular weight standards are indicated.*

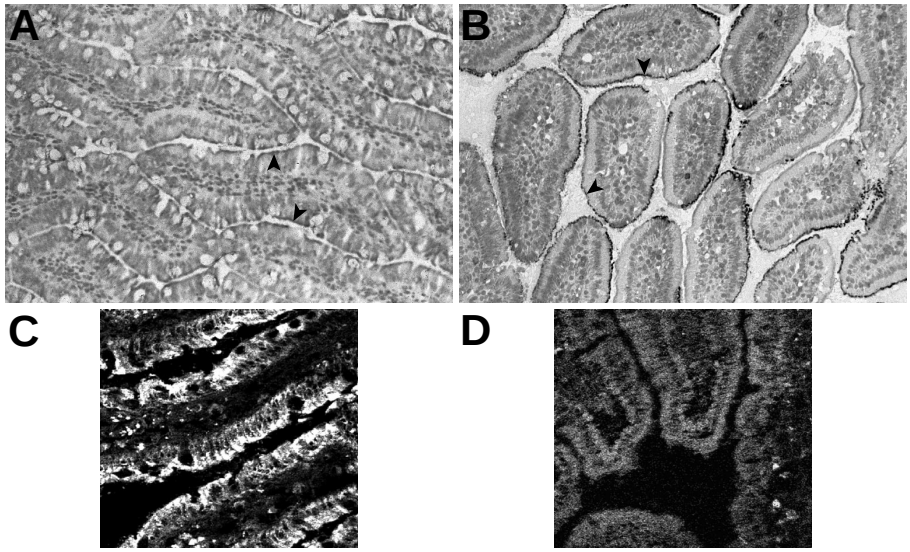

Figure 2*: Immunohistochemical (A, B) and confocal microscopy (C, D) of SR-BI in frozen small intestinal sections from rodents. A = control rat; B = control mouse; C = control rat; D = control rat (no anti-SR-BI added). The small intestinal sections shown are taken ~15 centimeters distal from the stomach. Arrow heads indicate the apical membrane staining of the enterocytes. The magnification used was 20x (A and B) and 40x (C and D).*

Immunohistochemistry showed specific SR-BI staining of the apical membranes of rat and mouse enterocytes (**Figure 2 A and B**). This apical membrane staining was found throughout the whole length of the intestine. Only enterocytes were stained: the abundant goblet cell were all negative. Confocal microscopy confirmed the immunohistochemistry data and showed a strong apical staining of enterocytes of rats, while some intracellular staining was also evident (**Figure 2 C**).

Western blot analysis of brush border membranes isolated from different parts of the rat intestine revealed that SR-BI is more abundant in proximal parts of the intestine than in distal parts (**Figure 3, compare lanes 2 and 3**). BBM isolated from mouse intestine also showed higher abundancy of SR-BI protein in proximal than in distal parts (**Figure 4, compare lanes 1 and 2**).

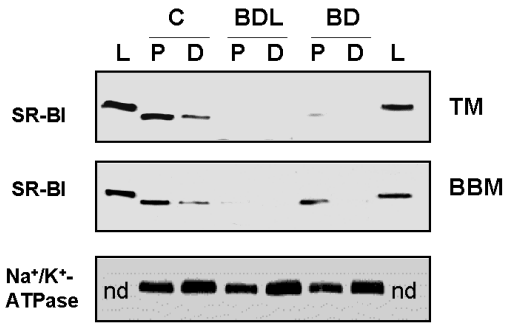

***Figure 3**: Representative Western blot of SR-BI in small intestinal total membrane (TM) and brush border membrane (BBM) fractions of control (C), bile duct-ligated (BDL) and bile-diverted (BD) rats. Proximal (P) and distal (D) parts of the intestine were used. Hepatic plasma membranes (L) were included as a positive control. The amounts of protein loaded was 1 mg for hepatic and 5 mg for intestinal preparations. Na^+/K^+-ATPase was used as a control signal (nd = not determined).*

Regulation of intestinal SR-BI expression by bile components: Protein levels of SR-BI in proximal and distal parts of the intestine were strongly reduced in bile duct-ligated rats (**Figure 3, lanes 4 and 5**) when compared to control rats (**Figure 3, lanes 2 and 3**) in both total membranes (TM) and brush border membranes (BBM). In bile diverted rats, the decrease in intestinal SR-BI levels was more pronounced when total intestinal membrane fractions instead of BBM were analyzed (**Figure 3, top panel**). In this case, only a weak band was found in the proximal intestine of bile-diverted rats. SR-BI levels decreased most pronouncedly in the BBM of the distal intestine to undetectable levels, whereas the proximal part showed only a minor decrease in comparison to controls. Bile duct-ligated mice also showed strongly reduced protein levels of SR-BI in the intestine (**Figure 4**). In contrast, *Mdr2*$^{(-/-)}$ mice displayed a relatively small decrease in SR-BI protein in the proximal part of the intestine, i.e., approximately by 40% compared to control mice (**Figure 4**).

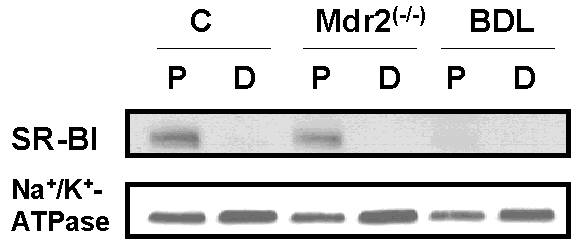

***Figure 4**: Representative Western blot of SR-BI in intestinal brush border membranes of control (C), Mdr2$^{(-/-)}$ and bile duct-ligated (BDL) mice. Proximal (P) and distal (D) parts of the intestine were used. The amounts of protein loaded was 10 mg. Na^+/K^+-ATPase was used as a control signal.*

To investigate whether the decrease in protein levels of SR-BI is mediated *via* transcriptional or post-transcriptional mechanisms, we evaluated steady state levels of intestinal SR-BI mRNA by RT-PCR in rats with manipulations of the biliary tract (**Figure 5**). The mRNA levels of HMG-CoA reductase (HMGR), a key enzyme in cholesterol synthesis, were used for comparison. HMGR mRNA was more abundant in distal parts of the intestine in control and bile duct-ligated rats (**Figure 5**). As expected, bile-diverted rats showed a strong increase of HMGR mRNA levels, which was particulary evident in the proximal intestine. In accordance with localization with of the SR-BI protein, SR-BI mRNA was most abundant in proximal

intestine of control rats (**Figure 5, lanes 1 and 2**). Steady state SR-BI mRNA levels were reduced by ~ 50% in the bile duct-ligated rats when expressed relative to b-actin mRNA (**Figure 5, bottom panel**). This decrease was statistically significant ($p < 0.05$) for the proximal part of the intestine only. Bile diverted rats did not show a significant change in SR-BI mRNA levels compared to the control rats. In a similar fashion, bile duct-ligated mice showed a strong reduction in SR-BI mRNA levels whereas *Mdr2*$^{(-/-)}$ mice did not show any change in mRNA levels compared to control mice (Figure 6)

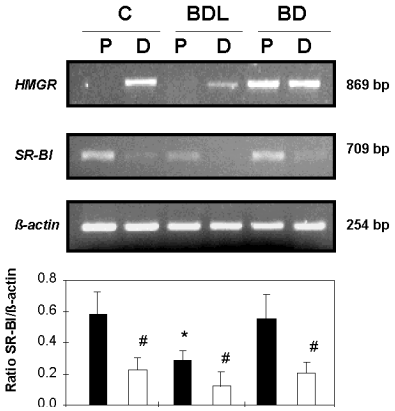

***Figure 5**: RT-PCR analysis of intestinal HMG-CoA reductase (HMGR) and SR-BI mRNA levels in control (C), bile duct-ligated (BDL) and bile-diverted (BD) rats. The mRNA levels of HMG), SR-BI and ß-actin shown represent a pool of three individual animals. The bottom panel shows relative amounts of SR-BI to ß-actin mRNA. The ratio between SR-BI and ß-actin mRNA was determined in three independent samples (mean – SD). (*) significantly different from control rats, $p < 0.05$; (#) significantly different from the proximal part of the intestine, $p < 0.05$ as determined by Mann Whitney U-test.*

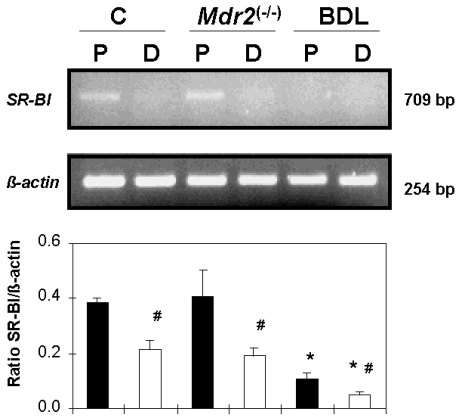

***Figure 6**: RT-PCR analysis of intestinal SR-BI mRNA levels in control (C), Mdr2$^{(-/-)}$ and bile duct-ligated (BDL) mice. The mRNA levels of SR-BI and ß-actin shown represent a pool of three different animals. The bottom panel shows relative amounts of SR-BI to ß-actin. The ratio between SR-BI and ß-actin were determined in three independent samples (mean – SD). (*) significantly different from control mice, $p < 0.05$; (#) significantly different from the proximal part of the intestine, $p < 0.05$ as determined by Mann Whitney U-test.*

DISCUSSION

The results of this study confirmed the presence of SR-BI in rat and mouse intestine and clearly demonstrate that the protein is present at the apical membrane of the enterocytes. Furthermore, we demonstrate that SR-BI is more abundant in proximal than in distal segments of the intestine, i.e., a distribution pattern that mirrors that of HGMR, the rate-limiting enzyme in cholesterol biosynthesis. Finally, intestinal SR-BI appeared to be down-regulated at post-translational and translational levels in both bile-deficient rodent models.

As previously described [19] the molecular weight of the protein bands recognized by the anti SR-BI antibody in intestinal brush border membranes of rats and mice was ~ 4 kDa lower than that found in the liver. Deglycosylation of hepatic plasma membranes and intestinal brush border membranes proteins yielded

bands at ~ 60 kDa, approaching the size of the native protein based on its amino acid sequence [21]. Whether intestinal SR-BI is indeed less glycosylated than hepatic SR-BI in the *in vivo* situation or whether it reflects an artifact due to the isolation procedure used is not known. Hauser *et al*. [20] reported a ~ 84 kDa protein in intestinal brush border membranes of rabbits, identical in size as adrenal rabbit SR-BI, using a similar isolation procedure. We further confirmed the presence of SR-BI in the brush border membrane by immunohistochemistry, demonstrating clear staining of the apical membrane of enterocytes. Apical membrane staining was found along the whole length of the small intestine, however, levels of SR-BI were much higher in proximal parts of the intestine than in distal parts. Since SR-BI has been implicated in intestinal cholesterol absorption [20], these results would be in accordance with the proximal absorption of dietary cholesterol, as reported by Arnesjö *et al*. [43] and Borgström [44] in humans. However, other reports [45-47] indicate absorption along the whole length of the intestine. Our RT-PCR data confirmed the more proximal expression of SR-BI, since steady state mRNA levels of SR-BI were approximately three times higher in the proximal than in the distal parts.

Intestinal SR-BI protein levels were reduced in both bile-deficient rat models, suggesting that bile components play a role in the regulation of intestinal SR-BI expression. The decrease was more pronounced in the total membrane fractions of bile-diverted rats than in brush border membrane fractions, which may indicate altered sorting of SR-BI in the intestinal cells, i.e., an accellerated recruitment of cellular SR-BI towards the apical membrane in the bile diverted rats. Confocal microscopy suggested that, in addition to the apical membrane, SR-BI is also present intracellularly in enterocytes of control rats.

Data presented in Figure 5 demonstrated that SR-BI down-regulation is exerted at a post-transcriptional level in bile-diverted rats, while in bile duct-ligated rats transcriptional events may contribute (Figure 5 and 6). Accumulation of bile components and/or cholesterol in the plasma could play a role in alternative regulation of SR-BI protein in the intestine of bile duct-ligated animals. This hypothesis is consistent with the finding that mRNA levels of HMGR are decreased in the intestine of bile duct-ligated animals. Accumulation of cholesterol in plasma and liver of cholesterol-fed rats has been shown to be associated with a decrease of hepatic SR-BI protein levels [48,49]. However, total cholesterol content in the intestine from the different animal models revealed no differences, indicating that intestinal cholesterol accumulation is not required to down-regulate SR-BI expression in these bile-manipulated rodent models.

In the rat models used we could not distinguish which of the bile components contribute to regulation of SR-BI in the intestine. To further investigate this issue, we determined intestinal SR-BI protein levels and mRNA levels in mdr2 P-glycoprotein-deficient mice. Results show that absence of biliary phospholipids and cholesterol reduces the protein levels of SR-BI in the intestine to a similar extent as seen in the bile-diverted rats without affecting steady SR-BI mRNA levels. Together, these results indicate that specifically the biliary lipids are specifically involved in the post-transcriptional regulation of SR-BI expression in the intestine. Further research, including intestinal infusion of bile components into bile-diverted rats, will give more insight in the specific role of bile components in the regulation of SR-BI in the intestine.

Intestinal cholesterol absorption was strongly decreased in both rat models with deficient bile delivery. At first sight, these data would support a relationship between the intestinal SR-BI protein levels and cholesterol absorption efficiency as suggested by Hauser *et al*. [20]. Data from Hauser *et al*. indicate that SR-BI protein would account for approximately 50% of the uptake of cholesterol, in brush border membrane vesicles, with a remaining ~ 50% accounted for by 'passive diffusion'. In bile duct-ligated animals and bile-diverted animals no bile is present in the intestine leading to complete absence of cholesterol solubilisation vehicles [7,12]. Protein levels of SR-BI are below detection limits in bile duct-ligated rats while in bile-diverted rats protein levels are only reduced by ~50%. Cholesterol absorption is decreased by > 95% in both models. These results suggest that absence of bile in the intestine mainly accounts for the strongly decreased intestinal cholesterol absorption. In *Mdr2*$^{(-/-)}$ mice, with no biliary phospholipid and cholesterol delivery into the intestine, cholesterol absorption was down by ~50% and protein levels of SR-BI were reduced by ~40%. In contrast, both bile duct-ligated (DM Minich, Thesis, University of Groningen, 1999) and bile-diverted rats [50] absorb up to 60% of dietary fats (non cholesterol fats) while control rats absorb ~ 92% of their dietary fats [50]. *Mdr2*$^{(-/-)}$ mice are capable to absorb > 95% of their dietary fat [51,52]. Thus, these results do not rule out a role of intestinal SR-BI in absorption of dietary cholesterol but, at the same time, indicate that a role in dietary lipid absorption, as also suggested by Hauser *et al*. [20], is highly unlikely.

In conclusion, we demonstrated the presence of SR-BI in the intestinal apical membrane of enterocytes of rats and mice and a reduction of SR-BI protein levels in two independent rat models and a mouse model of cholesterol malabsorption characterized by the absence of bile in the intestinal lumen and in a mouse model with cholesterol malabsorption due to the absence of biliary lipid secretion. Our data suggests that biliary lipids play may a role in the post-transcriptional regulation of SR-BI, which could include accelerated breakdown of the protein and/or lower translation efficiency. Under cholestatic conditions (bile duct-ligated rats and mice) accumulation of cholesterol in plasma may lead to transcriptional down-regulation of intestinal SR-BI expression. It should be noted, however, that the precise role of SR-BI in cholesterol absorption remains to be defined. The recently generated SR-BI knockout mouse [53] provides the model of choice to address this issue.

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